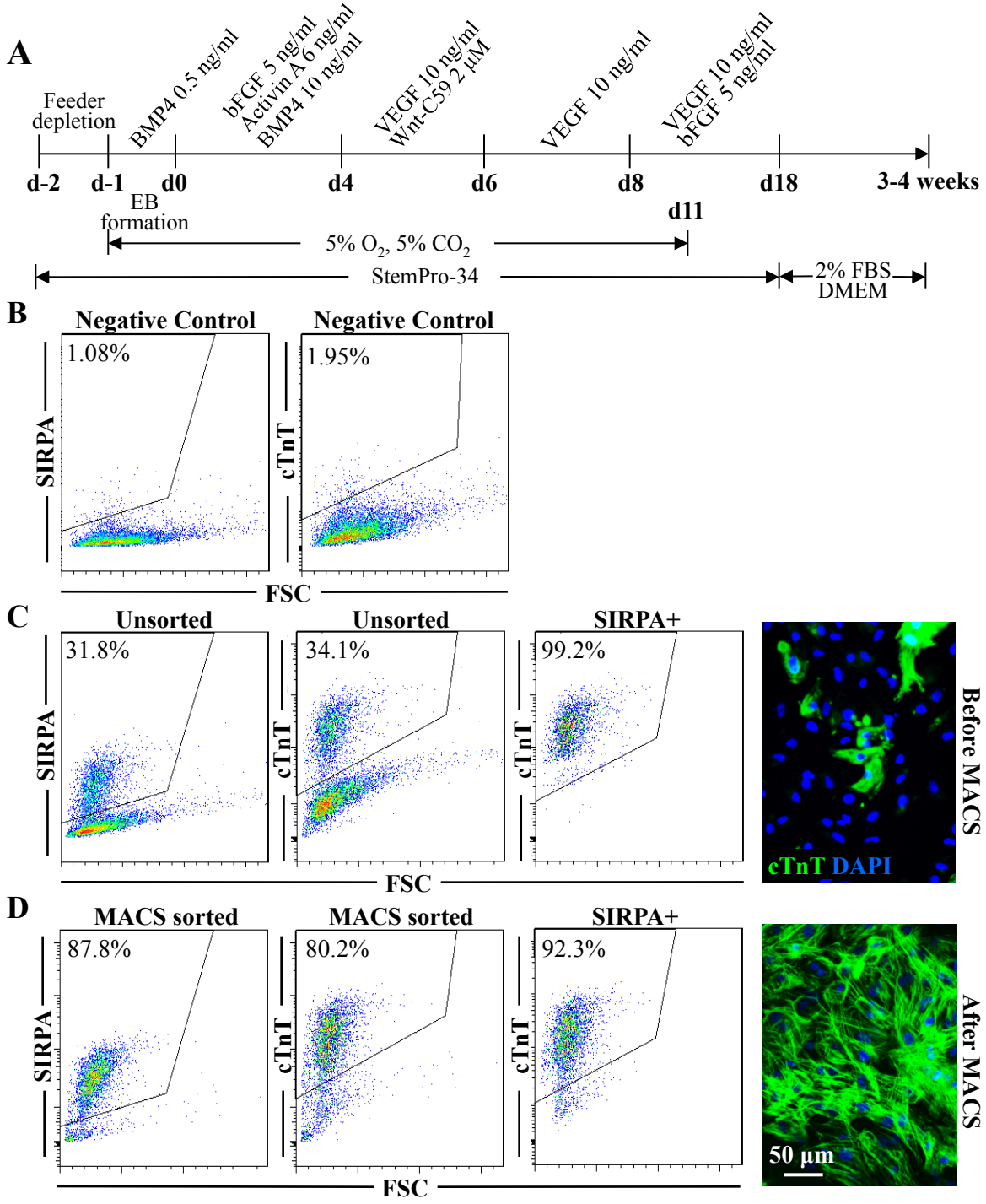
Supplemental Fig S1.

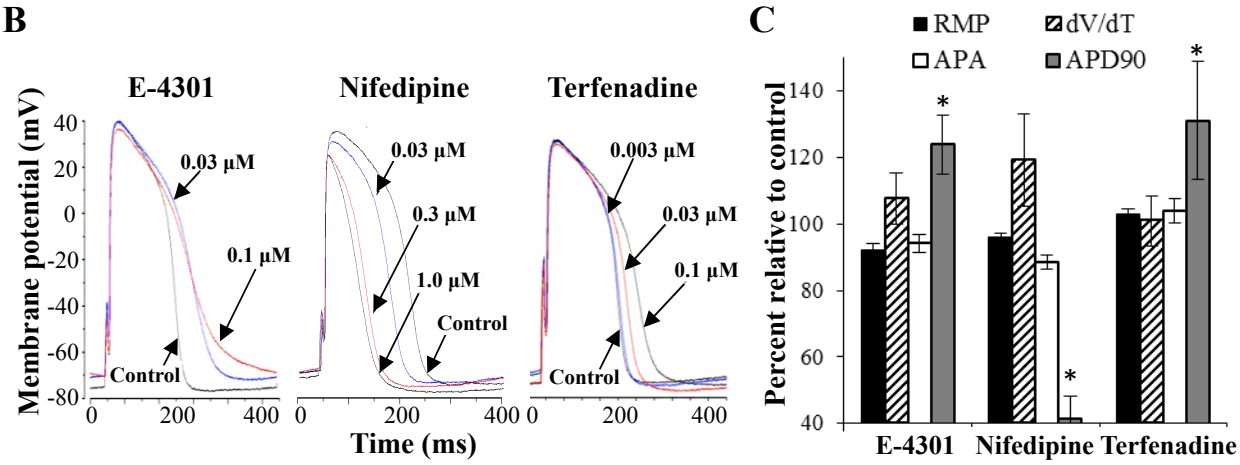


Supplemental Fig S1. Flow cytometry analysis of hESC-CM purity before and after MACS purification using SIRPA antibody. A) hESC cardiac differentiation protocol. After 3-4 weeks of differentiation, cells were purified/analyzed by MACS/FACS and used for patch and monolayer culture. B) Negative control PE and FITC isotypes. C) Pre-MACS flow cytometry analysis of SIRPA-PE (left), cTnT-FITC (middle), and cTnT in SIRPA gate (right). Immunostaining before MACS purification shows sparse $cTnT^+$ cells. D) Post-MACS flow cytometry analysis of SIRPA-PE (left), cTnT-FITC (middle), and cTnT in SIRPA gate (right). Immunostaining after MACS purification shows abundant $cTnT^+$ cells. Note that in unsorted and MACS purified cells, 92-99% of $SIRPA^+$ cells are also $cTnT^+$. FSC, Forward-scattered light. cTnT, cardiac troponin T.

Supplemental Fig S2.

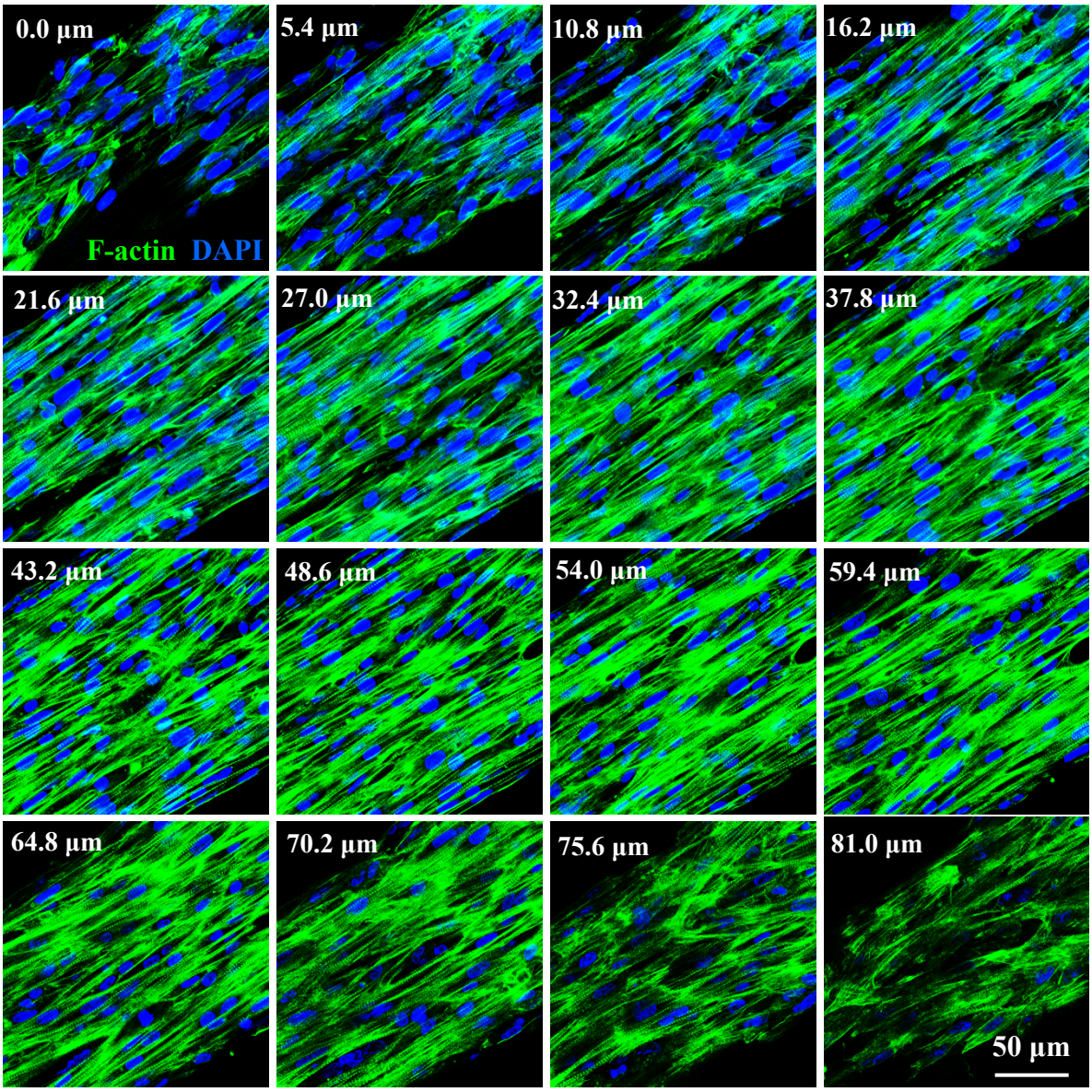
A

Control	APD_{30} (ms)	APD_{60} (ms)	APD_{90} (ms)	RMP (mV)	APA (mV)	$(dV/dt)_{max}$ (V/s)
Mean ± SEM (n= 79)	159.4 ± 7.1	195.5 ± 8.5	221.3 ± 8.8	-70.9 ± 0.5	102.9 ± 1.0	38.1 ± 1.5



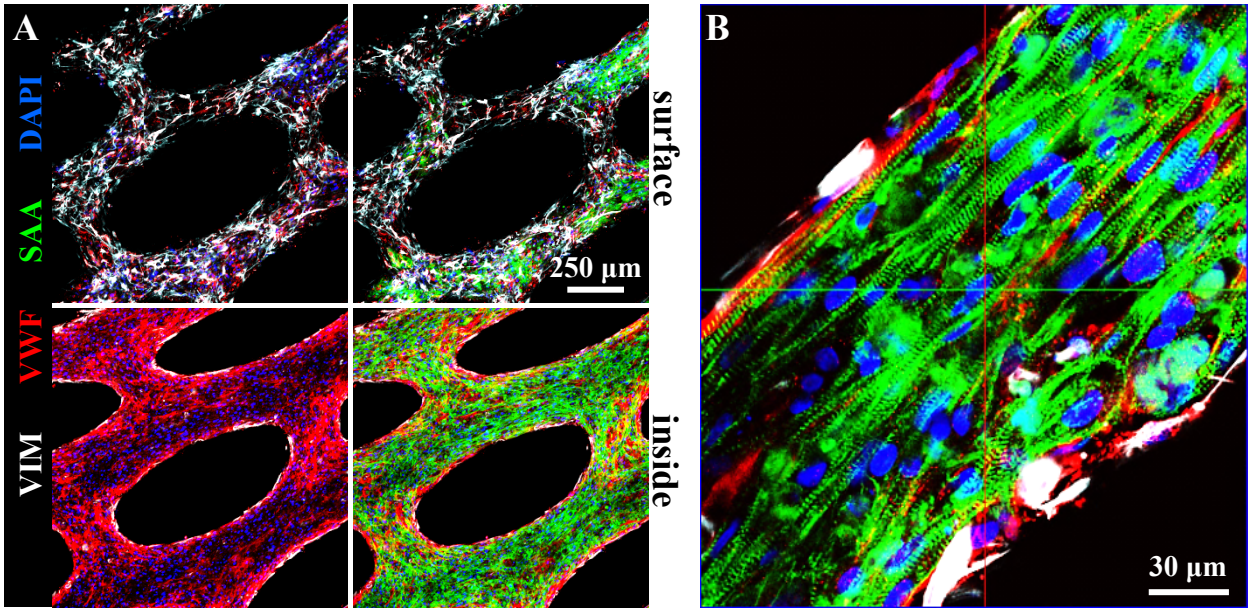
Supplemental Fig S2. Electrophysiological characteristics of hESC-CMs. A) Action potential (AP) properties of drug-free (control) hESC-CMs. B) Whole cell current-clamp traces of membrane voltage in hESC-CMs in the presence of different concentrations of HERG K^+ channel blocker E-4031 (left), L-type Ca^{2+} channel blocker nifedipine (middle), and ATP-sensitive K^+ channel blocker terfenadine (right). C) Quantification of resting membrane potential (RMP), AP upstroke velocity (dV/dt_{max}), AP amplitude (APA), and AP duration at 90% repolarization (APD90) in hESC-CMs treated with either E-4031 (0.1uM, n=7), nifedipine (1µM, n=6), or terfenadine (0.1uM, n=4) relative to vehicle control (100%); *p<0.05 compared with control.

Supplemental Fig S3.



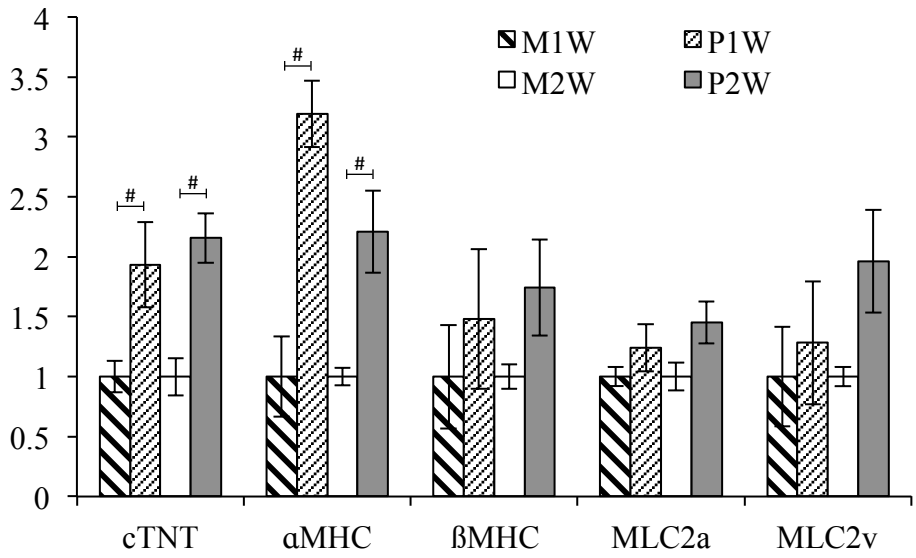
Supplemental Fig S3. Representative confocal image stack demonstrating uniform hESC-CM density and alignment throughout the thickness of a 2-week old cardiac tissue patch. Numbers in the top left corner denote imaging depth inside the patch. Green, F-actin; blue, DAPI. The patch was made of differentiated hESCs containing 70% hESC-CMs.

Supplemental Fig S4.



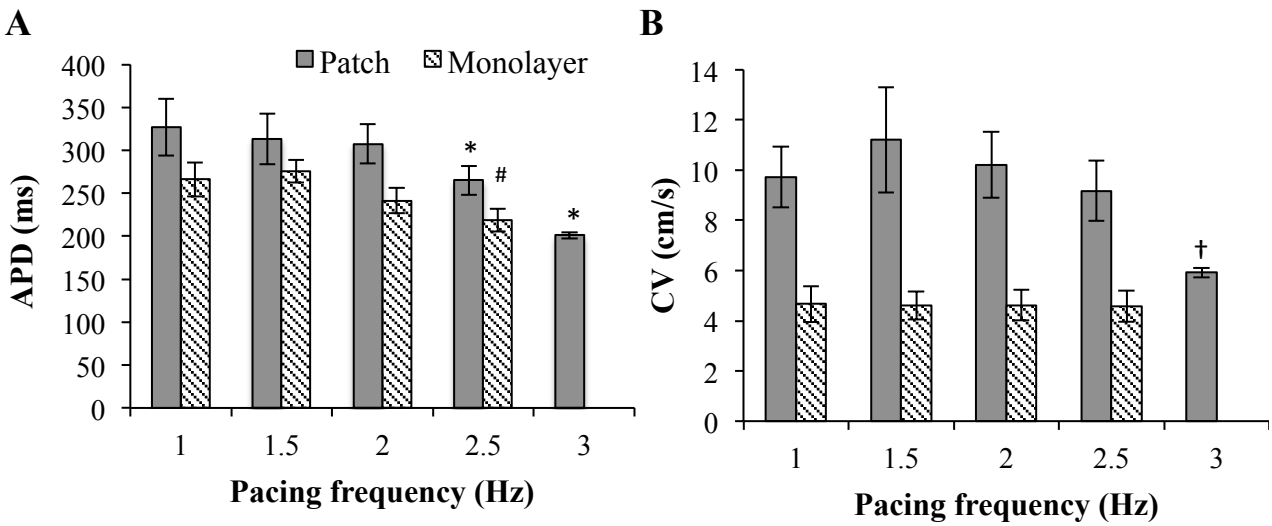
Supplemental Fig S4. Cellular composition of cardiac tissue patches. A) Representative immunostaining of a tissue patch made with 65% hESC-CMs showing that endothelial cells double-positive for von Willebrand factor (vWF) and Vimentin (Vim) were interspersed throughout the patch while Vim^{+}/vWF^{-} cells (fibroblasts) were mainly present on the patch surface. B) Sarcomeric alpha actinin (SAA)-positive cross-striated cardiomyocytes were aligned throughout the patch (also shown in top-right and bottom-right panels in A).

Supplemental Fig S5.



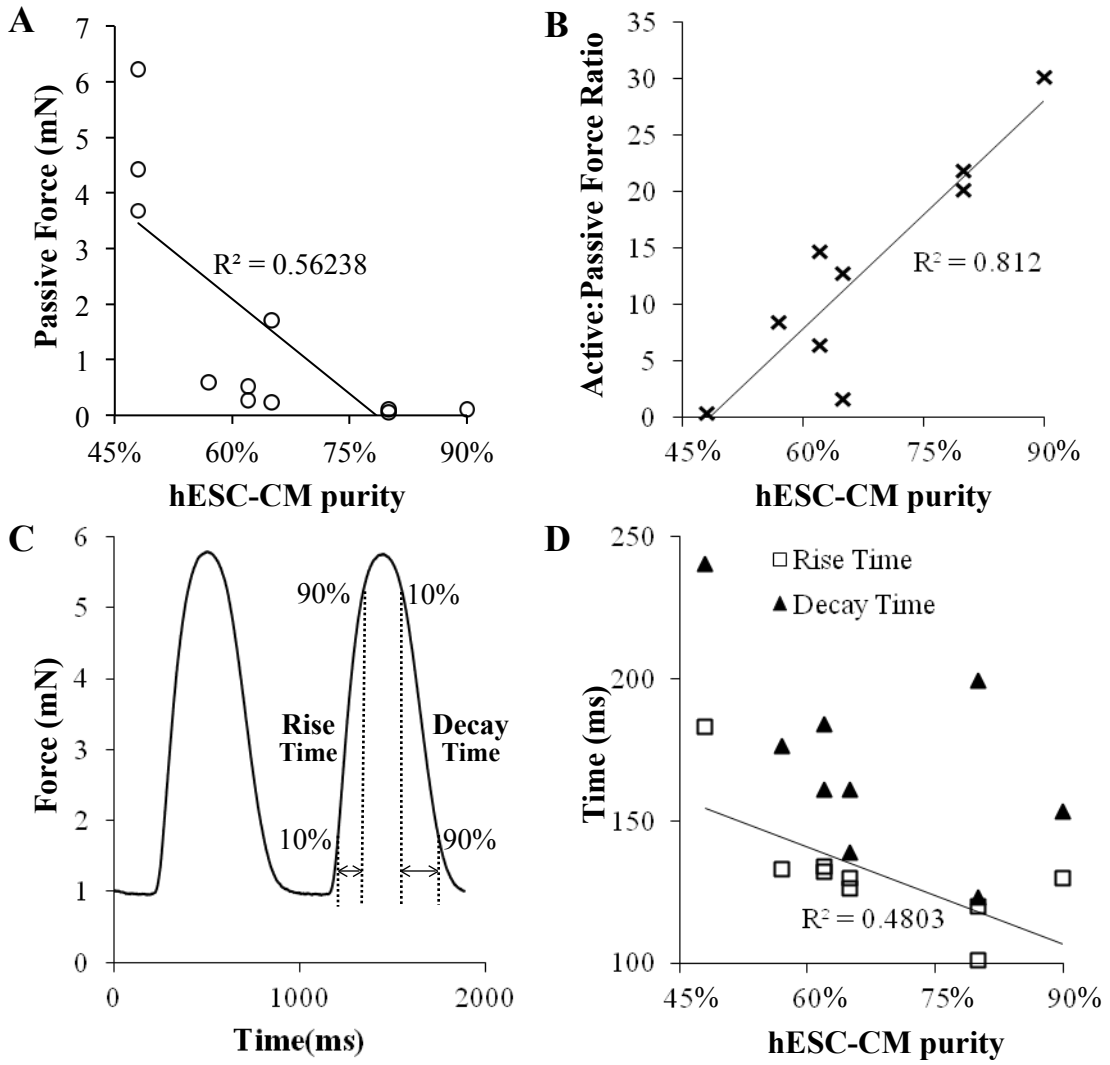
Supplemental Fig S5. Comparative expression of genes important for cardiac contractile function in age-matched 2D monolayers and 3D tissue patches. M1W and MW2, 1- and 2-week old monolayers; P1W and P2W, 1- and 2-week old tissue patches. cTnT, Cardiac troponin T; αMHC, alpha-myosin heavy chain; βMHC, beta-myosin heavy chain; MLC2a, myosin light chain-2 atrial; MLC2v, myosin light chain-2 ventricular. Gene expression levels in patches are shown normalized to those of age-matched monolayers. #, significant difference between patches and monolayers (n = 2-4, with 3 technical replicates). Monolayers and patches were made from differentiated hESCs containing 60-65% hESC-CMs.

Supplemental Fig S6.



Supplemental Fig S6. Electrical restitution relationships in 2-week old cardiac tissue patches and monolayers. A-B) Dependence of action potential duration (APD) and conduction velocity (CV) on pacing frequency. *$p<0.05$ relative to all other pacing rates. #$p<0.05$ relative to 1.5 and 2 Hz pacing rates; †$p<0.05$ relative to 1, 1.5, and 2Hz pacing rates. n = 4-5 monolayers, 5 patches between 1-2.5Hz) and 2 patches at 3Hz. Monolayers and patches were made from differentiated hESCs containing 48-65% hESC-CMs.

Supplemental Fig S7.



Supplemental Fig S7. Contractile properties of 2-week old cardiac tissue patches. A) Passive tension of tissue patches decreases with increased hESC-CM purity of cells used for patch production. B) Active:passive force ratio increases linearly with hESC-CM purity. Forces in A-B are measured at 10% stretch during 1Hz stimulation. C) Representative isometric twitch force traces in cardiac tissue patches (measured at 10% stretch and 1Hz stimulation. D) Rise and Decay Times of twitch traces (measured as shown in C) as a function of hESC-CM purity. While both Rise and Decay Times appear to decrease with increase in cardiomyocyte purity, only the Rise Time shows statistically significant trend (R^2=0.48, $p<0.02$).